T cell receptor signalling networks: branched, diversified and bounded

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Abstract | Engagement of antigen-specific T cell receptors (TCRs) is a prerequisite for T cell activation. Acquisition of appropriate effector T cell function requires the participation of multiple signals from the T cell microenvironment. Trying to understand how these signals integrate to achieve specific functional outcomes while maintaining tolerance to self is a major challenge in lymphocyte biology. Several recent publications have provided important insights into how dysregulation of T cell signalling and the development of autoreactivity can result if the branching and integration of signalling pathways are perturbed. We discuss how these findings highlight the importance of spatial segregation of individual signalling components as a way of regulating T cell responsiveness and immune tolerance.

Immunological synapse
The immunological synapse forms at the interface between the T cell receptor (TCR) and antigen-presenting cell and is traditionally characterized by a 'bull's eye' structure consisting of a central supramolecular activation cluster (cSMAC), a peripheral SMAC and a distal SMAC, creating the site at which TCR signal transduction is coordinated.

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T cells are key effectors of the adaptive immune response, with a number of important roles in the elimination of pathogens. They are also major effectors in autoimmune diseases and, therefore, there is an important need to understand how they are activated and regulated. Activation of T cells occurs upon ligation of clonotypic T cell receptors (TCRs) by MHC molecules on antigen-presenting cells (APCs) presenting peptides (peptide–MHC) from either endogenously encoded self molecules or exogenously encoded pathogen molecules. Precisely how TCR–peptide–MHC ligation results in the activation of the T cell, and its subsequent effect on proliferation and differentiation, to generate effector and memory immune responses, is poorly understood. We also lack an understanding of how immune responses are terminated to ensure that T cells return to a resting state and to limit pathology. Over the years our knowledge of the molecules involved in early TCR signalling has increased considerably and this has been aided by novel technologies, such as advances in imaging (BOX 1), which allow us to interrogate molecular interactions in a dynamic manner.

This Review focuses on some new aspects of TCR signal regulation that influence the way T cell responses are controlled. When T cells encounter antigenic peptide–MHC molecules on professional APCs in the lymph node, they stick, stop and activate a programme of proliferation and differentiation. These actions involve increased integrin binding, cytoskeletal rearrangements, coordinated production and mobilization of transcription factors, and changes in metabolism, in the responding T cell. The signalling pathways that direct these events originate from TCR engagement at the immunological synapse. Thus, the first signalling molecules that are activated following TCR ligation feed into a radiating and intricately branching network of signalling cascades that need to be coordinately regulated. In addition, it is equally important that this signalling network is terminated in a timely and robust manner. Recent evidence indicates that the localization and segregation of signalling molecules is crucial to the successful initiation of T cell activation and, just as importantly, to the termination of these responses and thus, the maintenance of immune tolerance.

Rather than attempt to provide a comprehensive overview of recent data on individual signalling cascades in T cells, this Review focuses on new data that address how interactions between different branches of the signal transduction network influence T cell activation, differentiation and the termination of responses. We aim to show that these examples support the view that T cell signal transduction is an intricately branching network, rather than a top-down signalling pathway. We emphasize the biological consequences for the cell and the potential for aberrant signalling to cause immune pathology. In addition, we bias the examples towards recent publications, but we also point the reader to a number of excellent, more comprehensive reviews on individual aspects of T cell signalling that provide extensive cover of the older literature.

TCR signal initiation

Issues: T cells are inherently self-reactive. T cells are selected by self-peptide–MHC complexes in the thymus and, therefore, have an inherent bias for self-reactivity. It was previously considered that, as a result of the

Box 1 | **New techniques for assessing proximal TCR signalling**

Super-resolution imaging

Numerous novel imaging techniques have been developed that enable the visualization of single molecules at high resolution (reviewed in REF. 62). For example, total internal reflection fluorescence microscopy (TIRFM) has been particularly insightful for investigating the immunological synapse.

The advantages over previous imaging techniques include better resolution of protein localization, the ability to assess the dynamics of the interactions between individual components of signalling complexes and the ability to assess their enzymatic activity.

Limitations include the somewhat artificial way in which the T cells are stimulated in order to achieve the narrow focal excitation that is required for modern imaging techniques such as TIRFM. T cell stimulation can be achieved by a limited range of ligands that are either plate-bound or linked to phospholipid bilayers by connections such as glycosyl-phosphatidylinositol (GPI) anchors. The mobility of both types of bound ligands is different from when the same molecules are expressed by an antigen-presenting cell (APC); plate-bound ligands are immobile, whereas the GPI-linked ligands are not constrained by interactions with the cytoskeleton and are therefore generally much more mobile. In addition, the length of time over which signalling is followed tends to be rather limited in these studies, generally minutes rather than the hours that we know are required for the full activation of the T cell.

Proteomics

A powerful means by which to monitor large-scale phosphorylation dynamics upon T cell receptor (TCR) ligation, using novel quantitative mass spectrometry-based techniques; for example, stable isotope labelling by amino acids in cell culture (SILAC) (reviewed in REF. 102).

SILAC offers advantages that include high coverage of the proteome, accurate quantification of the phosphoproteome and low technical error in sample preparation owing to the pooling of cell samples. Limitations include the high cost of the technique and the need for extensive cell culture in order to achieve complete incorporation of the isotopically labelled amino acids into the proteome. In addition, only two or three samples can be directly compared at one time.

Mass cytometry (commercially known as cyTOF)

A novel approach that couples flow cytometry with mass spectrometry (reviewed in REF. 103). This technology uses antibodies labelled with radioisotopes (typically lanthanide metals), which can be the same antibody clone as that used in conventional flow cytometry. Antibody-labelled cells are subjected to single cell atomic mass spectrometry, allowing the assessment of protein expression. The advantages over conventional flow cytometry include the ability to measure >36 parameters at any one time, compared with a maximum of 18 parameters by fluorescence cytometry, and this number is estimated to increase with time. No spectral overlap is detected from neighbouring isotopes, eliminating the need for compensation matrices to correct for such overlap, as is required in fluorescence cytometry. There is also no biological background when using lanthanide metals, therefore, there is no equivalent of 'autofluorescence', which is a common problem when using fluorescence cytometry.

Disadvantages include no option to recover or sort the cells after sampling, as the process is destructive to the cells. In addition, the system is expensive to set up.

Imaging flow cytometry

A new technology that combines flow cytometry with imaging[104]. This technique has the advantage over conventional flow cytometry of enabling spatial information to be generated in addition to multiparameter fluorescence information. For example parameters, such as colocalization of proteins, subcellular localization of proteins and cell–cell interactions can be assessed.

The advantage over conventional microscopy is that a large number of cells can be analysed, providing an opportunity for statistical analysis. However, the resolution of the images is limited compared with purely image-based techniques.

deletion of self-reactive T cells in the thymus, newly formed T cells that made it into the periphery would not be able to respond to self antigens. However, the actual scenario is not this straightforward and self-reactive T cells do exist in the periphery. Recent analyses using self-peptide-containing MHC tetramers have quantified self-reactive T cells and shown that a readily measurable number of naive T cells from normal individuals both bind to[1] and can be activated by self-peptide–MHC complexes (M. Davis, personal communication). Moreover, resting naive T cells continuously receive tonic signals from self-peptide–MHC molecules in the periphery[2], which, in combination with signals through the interleukin-7 (IL-7) cytokine receptor (reviewed in REFS 3,4), are essential for their survival.

Tonic signals
Induced by low-affinity engagement of the T cell receptor (TCR) by self-peptide–MHC complexes. Tonic signals are important for the maintenance and the homeostatic proliferation of T cells.

The SRC family kinase (SFK) members LCK (also known as p56-LCK) and FYN (also known as p59-FYN) are the first molecules to be activated following TCR engagement (reviewed in REFS 5–7), and SFKs are essential to provide the tonic signalling that is required for the survival of naive T cells[8]. Thus, LCK is constitutively active in naive T cells and maintains a basal level of phosphorylation on TCR-associated ζ-chains[9]. The threshold of T cell activation must be precisely set to ensure that naive T cells are not activated by self antigens but that they can respond to foreign-peptide–MHC complexes. A fine balance is maintained whereby the potential for self-reactivity is always present but is controlled in the peripheral T cell repertoire. The observation that autoimmune pathology is fairly rare shows that this self-reactive potential is rigorously controlled in normal individuals.

Mechanics: how T cells are activated. Precisely how the engagement of the TCR with an agonist-peptide–MHC complex tips the signalling balance in favour of T cell activation is still a matter of debate and has been extensively discussed elsewhere[10]. In this Review, we consider how SFKs initiate signalling and how the regulation of SFKs affects not only signal branching, but also signal termination, which is currently poorly understood.

The TCR has no intrinsic enzymatic activity and instead depends on the kinase activity of the SFKs, particularly LCK, to initiate signalling. It has long been known that LCK binds to the cytoplasmic domains of the TCR co-receptors CD4 and CD8 (REF. 11). Following interaction of the TCR with a peptide–MHC complex, these co-receptors are important for targeting the delivery of LCK into close proximity to its substrates: TCR-associated CD3 and ζ-chain immunoreceptor tyrosine-based activation motifs (ITAMs)[12]. A number of models have been proposed to explain TCR triggering; we summarize briefly below the two that are currently most popular.

The conformational change model suggests that binding of a peptide–MHC molecule alters the conformation of the TCR and initiates signalling. Although there is a lack of biophysical evidence for TCR structural changes per se on ligand binding, recent evidence has suggested alternative scenarios that are consistent with this model[13]. Live unstimulated T cells were shown to have the signature tyrosine, and leucine or isoleucine, residues of their CD3ε ITAMs buried in the lipid bilayer of the plasma membrane. On activation, these transmembrane domains are released from the lipid bilayer and become available as substrates for LCK[13]. It is unclear what triggers the dissociation of the CD3ε cytoplasmic domain, but transitional changes in the local lipid environment on TCR engagement[13], or mechanosensing of a torque exerted on the CD3ε chain by the TCRs binding to a peptide–MHC complex, have been postulated as possible causes[14].

The kinetic segregation model suggests that TCR signalling is triggered as a result of the TCR being partitioned into areas of the lipid membrane that are rich in LCK and that lack the transmembrane phosphatase CD45 (REF. 15). Numerous studies have confirmed that there is segregation of molecules at the immunological synapse, however, the reason behind this is unclear. Van der Merwe and colleagues[15] suggest that exclusion of CD45 occurs because the extracellular domain of this molecule is much larger than that of the TCR, the co-receptors or the co-stimulatory molecules, essentially forcing CD45 out of the closely apposed membranes at the immunological synapse. A recent study[16] took the reductionist approach of transducing embryonic kidney cells with components of the TCR signalling machinery in order to dissect the molecular aspects of the triggering of this receptor. This study confirmed the exclusion of CD45 from the area of the plasma membrane that forms the contact between the T cell and the APC. The authors concluded that this was not due to molecule size, but rather that the binding energy of the TCR to the peptide–MHC complex was sufficient to generate an exclusion force for transmembrane proteins with large or unligated extracellular domains[16], such as CD45.

Following triggering of the TCR, the abundance of LCK together with the abundance and location of its regulators dictate the extent to which the targets of LCK will be phosphorylated[17]. These targets include the tyrosine residues in the ITAMs of TCR-associated CD3γ chain, CD3δ chain, CD3ε chains and the ζ-chains, and the SYK family kinase ZAP70 (ζ-chain associated protein kinase of 70 kDa). Classically, the TCR 'signal triggering module', as coined by Acuto *et al.*[10], involves LCK-dependent phosphorylation of the ITAMs, allowing the recruitment and the LCK-dependent phosphorylation of ZAP70. This aggregation and phosphorylation results in conformational changes in ZAP70 (REF. 18), which promote its kinase activity, leading to the phosphorylation of its target molecules, including the key adaptor molecule linker for activation of T cells (LAT). In turn, the phosphorylated tyrosine residues of LAT recruit multiple downstream adaptor and signalling molecules, which activate several major signalling branches, with a number of consequences: upregulation of integrin affinity, which promotes cell adhesion; the coordinated mobilization to the nucleus of transcription factors that are crucial for the expression of genes necessary for T cell growth and differentiation; and actin reorganization, which is essential for T cell activation, proliferation and adhesion (FIG. 1). Together, these processes result in the proliferation and differentiation of T cells into effector T cells. If these signalling pathways are not properly coordinated, and if feedback loops are not activated to limit the effector response, there is a danger that tolerance will be breached, which would lead to immune pathology.

Activation of SFKs. The SFKs are positively regulated by phosphorylation of an activating tyrosine residue in their catalytic domain, which stabilizes an open conformation and promotes their full kinase activity. Conversely, they are negatively regulated by phosphorylation of a tyrosine residue in their carboxy-terminal domain, which results in a closed conformation and the downregulation of their kinase activity[19,20]. The activating tyrosine residue is autophosphorylated by the SFKs themselves and dephosphorylated by several candidate phosphatases, including CD45 and the cytosolic phosphatases protein tyrosine phosphatase non-receptor type 6 (PTPN6; also known as SHP1) and PTPN22 (for a review see REF. 21). The negatively regulating C-terminal tyrosine of the SFKs is phosphorylated by a single kinase, C-terminal SRC kinase (CSK), and dephosphorylated by CD45. In the absence of either of these molecules, LCK activity and the threshold of T cell activation are grossly affected: thymocytes in T cell-specific CSK-deficient mice display constitutive TCR signalling[22,23], whereas T cells lacking CD45 fail to initiate TCR signalling[24]. Therefore, CSK and CD45 can be considered to be the gatekeepers of TCR activation, and they regulate this activation by controlling the phosphorylation of the C-terminal negatively regulating tyrosine (Tyr505) of LCK.

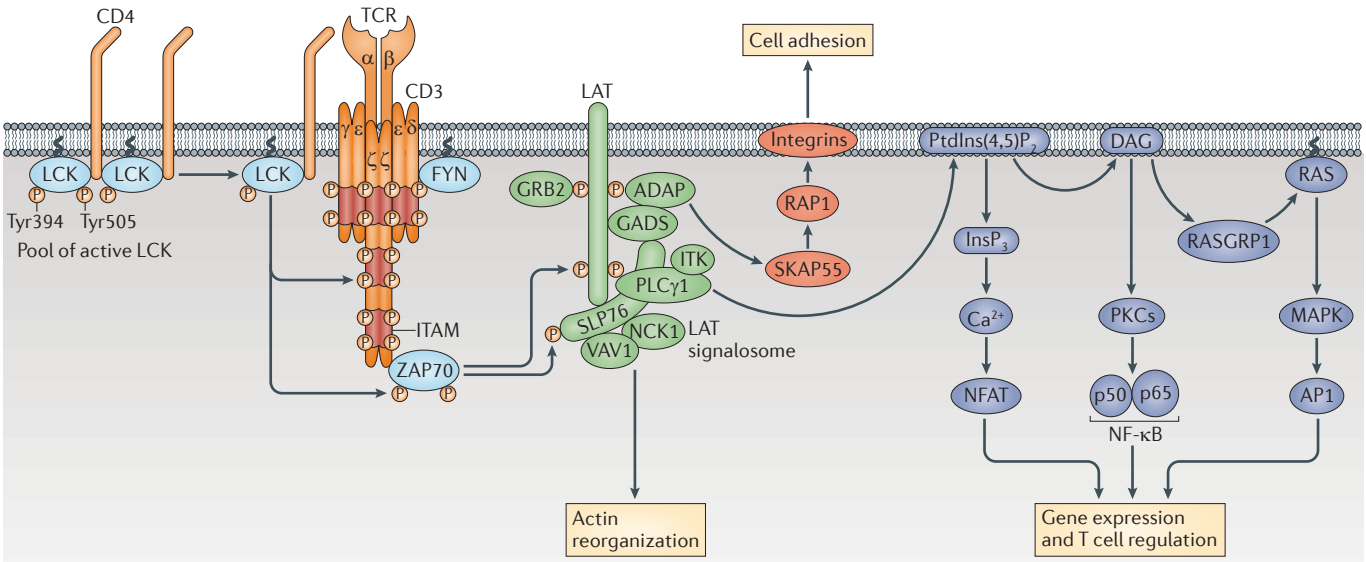

Figure 1 | **Overview of TCR signalling.** T cell receptor (TCR) signal transduction is initiated by the recognition of cognate peptide–MHC molecules. The first molecule to be recruited to the TCR–CD3 complex is the SRC family kinase (SFK) member LCK, which phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3γ chain, CD3δ chain, CD3ε chains and the ζ-chains. Phosphorylation of the ITAMs enables the recruitment of ZAP70 (ζ-chain associated protein kinase of 70 kDa), its phosphorylation by LCK and its activation. Activated ZAP70 phosphorylates four key tyrosine residues on linker for activation of T cells (LAT), which recruits numerous signalling molecules to form a multiprotein complex, termed the LAT signalosome. Important molecules that constitute this complex include phospholipase Cγ1 (PLCγ1), growth factor receptor-bound protein 2 (GRB2), GRB2-related adaptor protein GADS, SLP76 (SH2 domain-containing leukocyte protein of 76 kDa), adhesion- and degranulation-promoting adaptor protein (ADAP), interleukin-2-inducible T cell kinase (ITK), NCK1 and VAV1. The LAT signalosome propagates signal branching to three major signalling pathways, the Ca^{2+}, the mitogen-activated protein kinase (MAPK) kinase and the nuclear factor-κB (NF-κB) signalling pathways, leading to the mobilization of transcription factors that are critical for gene expression and essential for T cell growth and differentiation. Signals initiated from the TCR also result in actin reorganization and the activation of integrins by inside-out signalling. AP1, activator protein 1; DAG, diacylglycerol; $InsP_3$, inositol-1,4,5-trisphosphate; NFAT, nuclear factor of activated T cells; PKC, protein kinase C; $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; RASGRP1, RAS guanyl-releasing protein 1; SKAP55, SRC kinase-associated phosphoprotein of 55 kDa.

The abundance and location of CD45 (REFS 25,26) and CSK[27] in T cells are crucial in determining the threshold of activation. Recent phosphoproteomic analysis (BOX 1) shows that the overall level of phosphorylation of LCK is unchanged in resting T cells compared with activated T cells. Moreover, the same analysis showed that approximately 40% of LCK molecules are constitutively active and include a proportion that are phosphorylated on both the activating (Tyr394) and the negatively regulating (Tyr505) residues[28]. Therefore, the local concentration of active LCK molecules is likely to be more important than the overall phosphorylated state of the molecules in determining whether TCR signalling is triggered. In further support of this idea, a study using high-resolution imaging showed that LCK molecules expressed in Jurkat T cells cluster differently depending on their conformation; thus, LCK in the active, open conformation clusters after TCR triggering, potentially promoting autophosphorylation of the activating Tyr394 residue, whereas LCK in the closed, inactive conformation is prevented from clustering following triggering of the TCR[29]. However, the absence of CD4 or CD8 expression in Jurkat T cells makes it possible that the usual presence of these co-receptors in peripheral T cells would alter the mechanism of activation of LCK.

CSK is a cytosolic kinase that is recruited to the plasma membrane by membrane-associated adaptor molecules (reviewed in REF. 30), where it is juxtaposed with its SFK targets to mediate its inhibitory effects[31]. The proximity of CSK to LCK is important for maintaining the tonic signalling balance, and perturbations in the location of CSK can alter the dynamics of early TCR signalling. This was recently demonstrated in experiments in which the amino terminus of CSK was engineered to constitutively target CSK to the plasma membrane of Jurkat T cells[32]. A consequence of this constitutive localization was that the signalling threshold of the cells was raised so that they were refractory to TCR stimulation. Moreover, simply inhibiting the kinase activity of membrane-targeted CSK (by the introduction of a point mutation in the kinase domain that binds a specific small-molecule inhibitor), in the absence of any TCR triggering, resulted in the activation of LCK and the phosphorylation of its downstream targets[32]. Thus, the location and kinase activity of CSK influence the finely set threshold of T cell activation.

CSK recruitment to the plasma membrane. We do not fully understand how CSK is normally recruited to the plasma membrane in T cells. Various transmembrane adaptor molecules with the potential to interact with

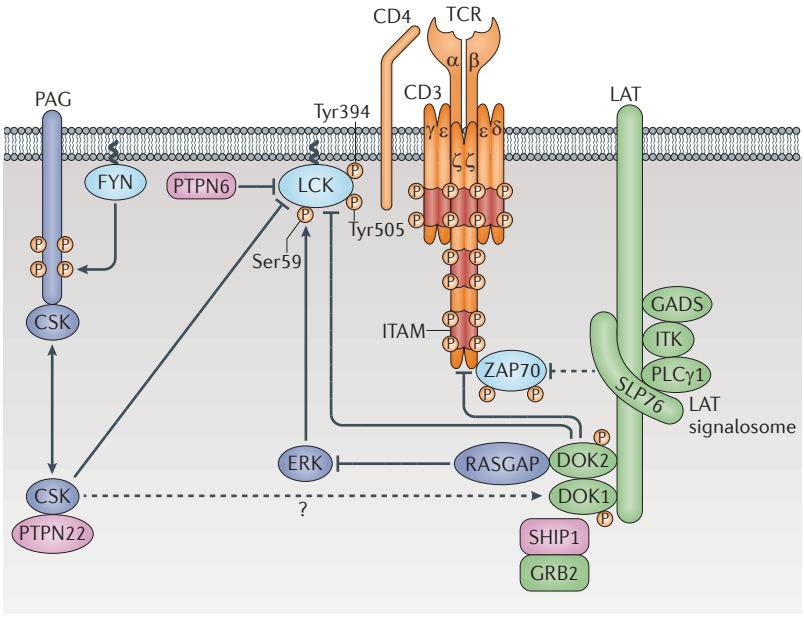

Figure 2 | **Negative feedback.** The phosphorylation status of LCK is dynamically regulated by a combination of autophosphorylation, the kinase CSK and the phosphatases CD45, protein tyrosine phosphatase non-receptor type 22 (PTPN22) and PTPN6. LCK is found in T cells in three forms: phosphorylated only on Tyr505 (inactive), phosphorylated only on Tyr394 (active) or phosphorylated on both Tyr394 and Tyr505 (active). The negatively regulating Tyr505 of LCK is phosphorylated by CSK and dephosphorylated by CD45 (not shown). The activating Tyr394 is autophosphorylated by LCK (or phosphorylated by FYN) and dephosphorylated by CD45, PTPN22 or PTPN6. These actions are likely to be controlled by the correct juxtaposing of these molecules in the vicinity of the T cell receptor (TCR). CSK is recruited from the cytoplasm to the cell membrane by its interaction with phosphorylated adaptor molecules such as phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG) or downstream of kinase 1 (DOK1). CSK binds to PTPN22, probably when both are in the cytosol, rather than when CSK is bound to membrane adaptor molecules. Upon TCR ligation, PAG is dephosphorylated by FYN, enabling the release of CSK and relieving LCK of the negative regulation imposed by Tyr505 phosphorylation. TCR stimulation triggers the rapid phosphorylation of DOK1 and DOK2, which form a complex and interact with linker for activation of T cells (LAT). DOK1 and DOK2 negatively regulate TCR signalling by recruiting further negative regulators such as RAS GTPase activating protein (RASGAP), SH2 domain-containing inositol polyphosphate 5′ phosphatase (SHIP1) and possibly CSK. RASGAP inhibits RAS activation leading to extracellular signal-regulated kinase (ERK) downregulation. ERK activity positively feeds back to LCK by phosphorylating Ser59, which is thought to block the interaction between LCK and PTPN6. SLP76 (SH2 domain-containing leukocyte protein of 76 kDa) might exert another level of negative feedback by impairing the clustering of ZAP70 (ζ-chain associated protein kinase of 70 kDa) molecules. GADS, GRB2-related adaptor protein; GRB2, growth factor receptor-bound protein 2; ITAM, immunoreceptor tyrosine-based activation motif; ITK, interleukin-2-inducible T cell kinase; PLCγ1, phospholipase Cγ1.

CSK are expressed by T cells. One of these is the ubiquitously expressed transmembrane adaptor molecule phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG; also known as PAG1 and CBP)[33].

Following T cell stimulation, PAG, which is kept phosphorylated by FYN in resting cells, becomes dephosphorylated (possibly by CD45 (REF. 34)) and can recruit CSK to the plasma membrane via its SRC homology 2 (SH2) domain, whereupon the activities of both LCK and FYN are downregulated[34]. Surprisingly, germline deletion of *Pag* has no obvious phenotype for T cell differentiation or activation[35,36]. The ability of LCK to be phosphorylated on its negatively regulating Tyr505 residue is undiminished in PAG-deficient mice, indicating that compensatory mechanisms which recruit CSK to the plasma membrane must be operating in these mice. Consistent with this, small interfering RNA (siRNA)-mediated depletion of *PAG* in mature human T cells was shown to enhance TCR stimulation, supporting a role for PAG in recruiting CSK to the plasma membrane, where it can act as a negative regulator[37] (FIG. 2).

New data indicate that CSK is also recruited to the plasma membrane by the cytosolic adaptor molecules DOK1 (downstream of kinase 1) and DOK2 (REF. 32), which have previously been shown to be negative regulators of T cell signalling[38,39]. DOK1 associates with CSK via its SH2 domain and becomes phosphorylated when the kinase activity of CSK is inhibited[32]. T cells deficient in DOK1 and DOK2 have prolonged phosphorylation of their TCR-proximal signalling molecules, such as the TCR ζ-chain and ZAP70. This indicates that there is enhanced LCK activity and results in increased T cell proliferation and cytokine secretion[38,39]. Therefore, the DOK adaptor molecules might have a role in limiting SFK signalling to maintain the threshold of activation and to limit the duration of signalling, which, if perturbed, has the potential to break immune tolerance and induce pathology.

TCR signal propagation

In addition to regulating the threshold of T cell activation, the duration and the location of SFK activation influences which downstream signalling cascades are activated, which affects the differentiation of the T cell and whether the response is appropriately controlled.

SFK signalling influences signal branching. An important illustration of how the regulation of SFK signalling influences the differentiation of cells comes from studies of fibroblasts. SFK activity in fibroblasts is limited in response to growth factor signalling by the recruitment of CSK to the plasma membrane by PAG. In fibroblasts, the phosphatase SHP2 (also known as PTPN11) dephosphorylates PAG, thereby releasing CSK from the plasma membrane; the SFKs rephosphorylate PAG, thereby recruiting CSK back to the plasma membrane[40].

This feedback loop influences the extent to which the SFKs propagate downstream signals, and, in particular, leads to RAS activation on endomembranes of the Golgi apparatus and endoplasmic reticulum, rather than at the plasma membrane[40]. Endomembrane-localized RAS is implicated in the sustained activation of the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK)[41,42] and in the activation of specific substrates, such as JUN N-terminal kinase (JNK) and AKT. By contrast, plasma membrane-localized RAS activity is thought to be more transient[42]. These physically segregated MAPK signalling modules integrate into different signalling pathways with distinct consequences for the control of cellular differentiation and proliferation[43].

Similarly, the extent of SFK activity in T cells influences the activity of phospholipase Cγ1 (PLCγ1), which in turn favours RAS activation on the Golgi apparatus, through the activation of the RAS guanine nucleotide-exchange factor (GEF) RAS guanyl-releasing protein 1 (RASGRP1)[41]. Ca^{2+} and diacylglycerol (DAG) positively regulate the translocation of RASGRP1 to the Golgi membranes, whereas they negatively regulate plasma membrane-associated RAS activity through the calcium-promoted RAS inactivator (CAPRI; also known as RASA4)[44], a GTPase-activating protein[41]. In Jurkat T cells, low-grade TCR stimulation leads to RAS activation on Golgi membranes[45], as do low-grade signals that promote the positive selection of developing thymocytes[46]. By contrast, stronger negatively selecting signals in thymocytes target RAS signalling to the plasma membrane[46], probably through the activation of growth factor receptor-bound protein 2 (GRB2)–SOS[41]. Thus, the quality and/or the strength of the TCR engagement influence the propagation of signals to different branches of the signalling cascade, which in turn affects the differentiation of T cells.

Signalosomes: key players in signal transduction. Most of the molecules with enzymatic properties in a signalling cascade, including kinases and phosphatases, contain domains that recruit other molecules and thereby regulate their localization. Important amino acids are targets of enzymatic activity and are transiently modified during signalling. For example, phosphorylated tyrosine residues act as docking sites for SH2 domains, thus joining different branches of the signalling network. Therefore, there is considerable potential for linear signal transduction cascades to branch into each other. In addition, specialized adaptor proteins function to colocalize with signalling molecules. The concept of the signalosome was adopted a number of years ago to describe the spatiotemporal recruitment of individual signalling components that are important for regulating different signalling cascades. Recent data (discussed below) have shown that, when signalosomes fail to form, T cell activation can go awry and result in fatal immune pathology.

The adaptor molecule LAT is the archetypal example of a protein that recruits other molecules to form a signalosome to facilitate T cell signalling. LAT is phosphorylated on several tyrosine residues, which in turn act as docking sites for kinases and other adaptor molecules (reviewed in REF. 47) (FIG. 1). By coordinating the recruitment of enzymes and their substrates to the TCR-proximal plasma membrane, LAT is considered to be a gatekeeper of T cell signalling that ensures signalling progresses in a regulated manner, thereby optimizing gene transcription and integrating other pathways that are involved in integrin activation and cytoskeletal rearrangements. Further reinforcement of the importance of LAT in the propagation of TCR signals comes from observations that cells deficient in LAT, such as the Jurkat cell mutant JCAM2.5, cannot propagate TCR signals[48], and that LAT-deficient mice fail to develop T cells past the $CD4^+CD8^+$ (double-positive) stage of thymocyte differentiation[49].

More recent studies have shown, surprisingly, that the LAT signalosome is not absolutely essential for the activation of peripheral T cells per se, but rather that it is essential for their effective and regulated activation. Neither a point mutation in the crucial Tyr136 residue of LAT that recruits PLCγ1 to the signalosome, nor the inducible deletion of LAT in mature T cells after they have completed thymic selection, prevents T cells from becoming activated *in vivo*[50].

Following transfer into immunodeficient hosts, LAT-mutated or LAT-deficient peripheral T cells show serious defects in their capacity to control their activation status and therefore become pathogenic, a syndrome termed LAT-signalling pathology[50,51]. Induction of LAT-signalling pathology requires the triggering of LAT-mutated cells through both the TCR and the co-stimulatory molecule CD28, although sustained proliferation and effector functions are subsequently driven by cytokine signalling[52]. In both LAT-deficient and LAT-expressing T cells, TCR engagement induces the activation of LCK and ZAP70, as well as that of an overlapping range of tyrosine-phosphorylated substrates, which indicates that LAT-independent signalling pathways exist[53]. The subsequent hyperproliferation of LAT-deficient T cells indicates that disruption of the LAT signalosome results in the loss of a negative regulatory loop that is required for signal termination, perhaps through the loss of recruitment of the negative regulatory adaptor molecules DOK1 and DOK2 to LAT[38,39] (FIG. 2). A partial loss-of-function (Tyr136Phe) mutation in LAT causes LAT-signalling pathology that is as severe as in the case of total loss of LAT expression, which indicates that the negative regulatory loops depend on the appropriate assembly of the intact LAT signalosome[53]. The unrestrained proliferation and cytokine production exhibited by LAT-deficient T cells illustrates the potential for clinical harm if T cell signalling is inappropriately regulated.

The observations that LAT is essential for effective and regulated T cell triggering make it necessary to re-evaluate our understanding of how the LAT signalosome operates in TCR signalling. In particular, it highlights the redundancy that can operate during TCR signalling, as LAT-deficient T cells can proliferate and produce cytokines, and therefore can activate much of the transcriptional machinery required for the expansion and the effector function of the T cell population. Although not excluding a role for LAT in PLCγ1 activation and the increase in Ca^{2+} levels, recent findings have shown that ERK can be activated in a LAT-independent manner — involving a complex of BAM32 (B cell adaptor molecule of 32 kDa; also known as DAPP1), PLCγ1 and PAK1 (p21-activated kinase 1)[54] — and that the key T cell adaptor molecule SLP76 (SH2 domain-containing leukocyte protein of 76 kDa) is still phosphorylated in LAT-deficient T cells[50].

SLP76 is phosphorylated by ZAP70, forming a complex with phosphorylated LAT via the GRB2-related adaptor protein GADS (also known as GRAP2)[55], and was originally considered to be downstream of LAT in the signalling cascade. SLP76 interacts with PLCγ1 and colocalizes with PLCγ1 and interleukin-2-inducible T cell kinase (ITK; a kinase important for intracellular

LAT-signalling pathology
An autoimmune lymphoproliferative disorder that results in excessive amounts of T_H2 cytokines and polyclonal B cell activation with hypergammaglobulinaemia (IgG1 and IgE), which is caused by mutations in key linker for activation of T cells (LAT) tyrosine residues or in the absence of LAT.

Ca^{2+} mobilization). SLP76 also regulates actin polymerization in response to TCR stimulation by bringing the RHO-family GTPase exchange factor VAV1, the adaptor protein NCK1 and actin nucleation-promoting factors, such as the Wiskott–Aldrich syndrome family proteins, into proximity. Furthermore, SLP76 can signal in the absence of LAT in platelets[56], which supports the idea that alternative routes for SLP76 activation exist when LAT is not present. Thus, SLP76 is an important adaptor molecule for regulating signalling between LAT and the actin cytoskeleton. Unlike LAT, SLP76 does not have a transmembrane domain and it is unclear how it is targeted to the immunological synapse in the absence of LAT.

The rather simplistic idea of TCR signalling progressing in a linear manner through the LAT signalosome needs to be re-evaluated. Clearly, an important feature of T cell signalling that is absent in LAT-deficient T cells is the ability to cease proliferation, and to die or to differentiate into relatively indolent memory T cells. Thus, the coordination of signalosomes is important for the termination of signalling and the maintenance of immune tolerance.

Spatiotemporal control of TCR signalling

Signalling microclusters and LAT recruitment. Early imaging studies indicated that, after antigen engagement, TCRs move to the centre of the ring-shaped immunological synapse, termed the central supramolecular activation cluster (cSMAC), where they initiate signal transduction. By contrast, the integrin LFA1 (also known as αLβ2 integrin) accumulates in an outer ring around this, which is known as the peripheral SMAC (pSMAC)[57,58]. More recently, real-time high-resolution imaging has shown that, rather than forming a solid ring of molecules, TCRs form dynamic TCR microclusters within seconds of antigen binding[59,60], and it is from these microclusters that proximal signalling is initiated[61]. Microcluster formation is distinct from and precedes the formation of the cSMAC, which is now thought to be the area where TCRs coalesce following protracted signalling, and from where they are internalized and degraded[61]. Understanding how microclusters interact is therefore crucial to appreciating how T cells become activated, whereas the degradation of signalling molecules is important in controlling how T cell signalling is terminated.

As LAT coordinates the appropriate propagation of TCR signalling, it is important to understand where it originates from and how it reaches the TCR to join these TCR microclusters, and this has become an area of debate in recent years (reviewed in REF. 62). The existing view that ZAP70 phosphorylates plasma membrane-bound LAT, which in turn recruits and phosphorylates the adaptor proteins GADS, SLP76 and other molecules in a linear manner, is almost certainly an over-simplification. By using super-resolution imaging to facilitate the visualization of single molecules in a cell, novel models have been proposed to explain how LAT and TCRs come together to form microclusters. One such model proposes that, rather than mixing freely in the plasma membrane, TCRs and LAT molecules form distinct 'protein islands' that fuse together to form a signalling microcluster on TCR engagement with a peptide–MHC molecule[63] (FIG. 3a). However, another study does not support the recruitment of these relatively large protein islands of LAT to the TCR and instead indicates that LAT might exist in the plasma membrane of both resting and active cells as nanoclusters, which may be as small as dimers[64]. In contrast to other studies, these nanoclusters are observed in resting and active cells — both separately, as pools of LAT alone, and as mixed pools, together with TCR molecules. Following TCR stimulation, these nanoclusters are found to increase slightly in size, to signal without the need for aggregation and to display a distinct ring of SLP76 at their periphery[64]. The presence of SLP76 at the periphery is dependent on actin polymerization and indicates that the nanoclusters might have a structure. Of note, the authors propose that there is a range of other signalling molecules associated with LAT nanoclusters, with GRB2 thought to be recruited to every nanocluster, whereas PLCγ1 has only been detected in certain nanoclusters. This proposal suggests a way in which the TCR signals can be directed along a particular branch of the signalling cascade depending on which molecules constitute the LAT nanocluster, thus promoting further signal diversification (FIG. 3b).

An alternative and distinct model indicates that, following TCR stimulation, LAT might be recruited to the TCR from sub-synaptic intracellular vesicles, rather than from the plasma membrane, and might be phosphorylated by ZAP70 at the activated TCR[65,66]. After phosphorylation, vesicle-associated LAT is recruited to SLP76- and GADS-containing microclusters, which are associated with the plasma membrane, resulting in the transient immobilization of these LAT-containing vesicles at the plasma membrane. An important corollary of this model is that it makes the order of the activation of molecules in the signalling cascade uncertain, as it is conceivable that the phosphorylation of SLP76 precedes that of LAT and, furthermore, occurs in a LAT-independent manner. As described above, the consequences of SLP76 activation without LAT signalosome assembly are uncontrolled T cell activation and immune pathology[50]. Precisely how SLP76 associates with the plasma membrane, if not via LAT, remains to be established but it is thought to involve a process of actin polymerization through interactions with the effector proteins NCK1, VAV1 and Wiskott–Aldrich syndrome protein (WASP)[67,68] (FIG. 3c).

The order in which TCR microclusters are assembled, and whether interactions occur solely at the plasma membrane, or between membrane-associated molecules and sub-synaptic vesicles, remains to be established. A potential outcome of the different localizations of signalling molecules could be to alter which signalling pathways are activated, as described above for the compartmentalization of RAS signalling. Such alterations could affect the differentiation pathway adopted by a stimulated T cell and might also negatively affect how the T cell response is controlled.

Advances in imaging — relevance to TCR triggering. Recent advances in imaging techniques (reviewed in REF. 62) (BOX 1) have greatly improved our ability to visualize both the range of different molecules that are

Cental supramolecular activation cluster
(cSMAC). After T cell receptor (TCR) engagement, the TCRs accumulate into a cluster at the interface between the T cell and antigen-presenting cell, termed the cSMAC. The cSMAC is surrounded by a ring of LFA1 that constitutes the peripheral SMAC. The most external ring is called the distal SMAC that is rich in large proteins, such as CD45, and in actin.

TCR microclusters
TCR microclusters are generated at the initial contact region of the T cell receptor (TCR)–antigen-presenting cell (APC) interface in the first minute of TCR engagement, before formation of the full immunological synapse. They consist of approximately 30–300 TCRs together with signalling molecules, and function as a minimal signalling unit, which translocates to the centre of the TCR–APC interface to form the central supramolecular activation cluster of the immunological synapse.

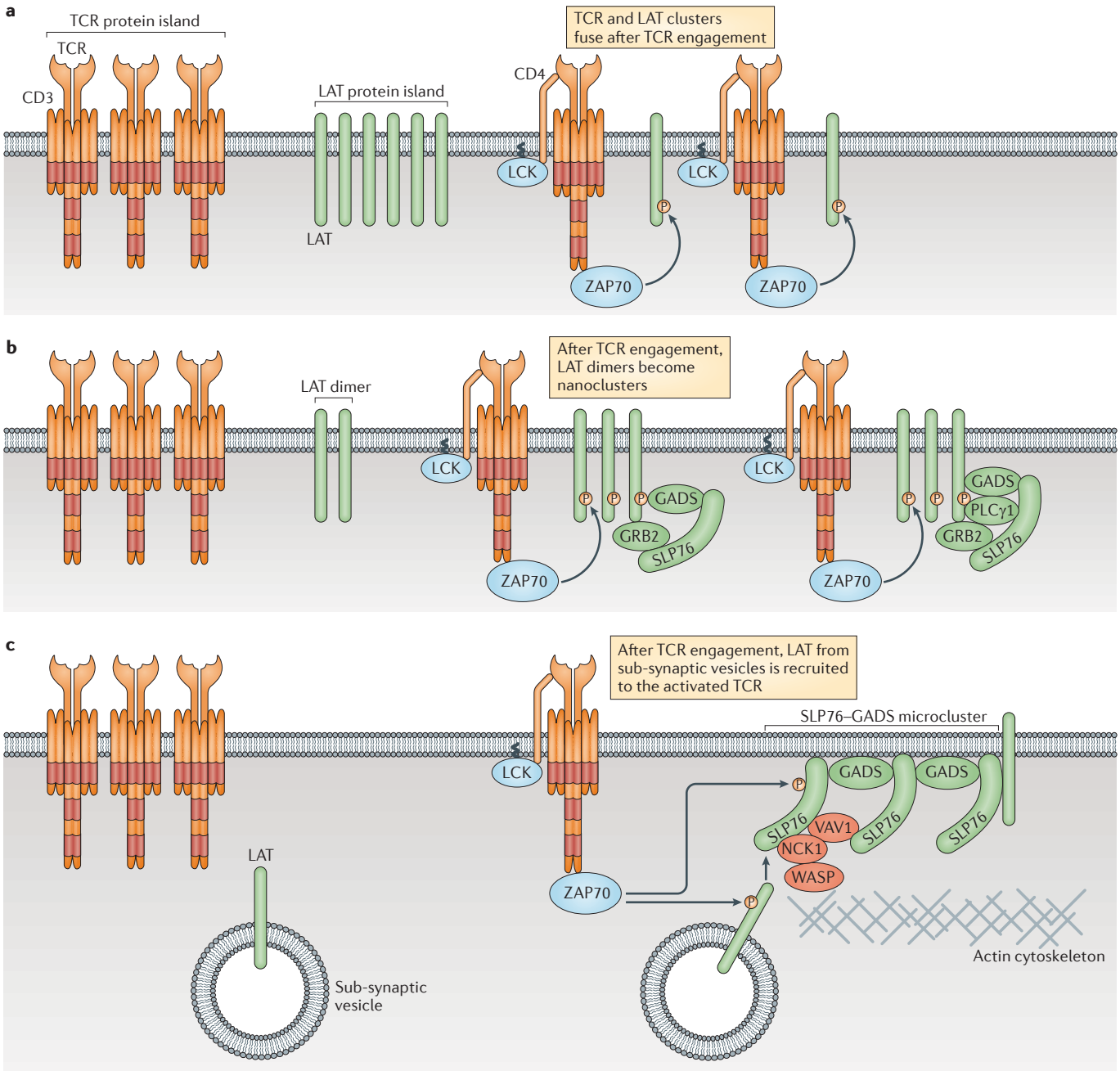

Figure 3 | **Key concepts in spatiotemporal control of LAT.** Proposed models for how linker for activation of T cells (LAT) reaches the T cell receptor (TCR). **a** | Relatively large protein islands of the TCR complex and LAT molecules exist as concatemers within separate protein islands in the plasma membrane in naive T cells. After TCR engagement, the protein islands fuse, forming a signalling microcluster. **b** | In both resting and active T cells, LAT exists in the plasma membrane as nanoclusters, possibly as dimers. After TCR stimulation these nanoclusters increase slightly in size and become phosphorylated by ZAP70 (ζ-chain associated protein kinase of 70 kDa) without the need for aggregation into larger microclusters. SLP76 (SH2 domain-containing leukocyte protein of 76 kDa) via growth factor receptor-bound protein 2 (GRB2), GRB2-related adaptor protein GADS, and phospholipase Cγ1 (PLCγ1) are recruited to these nanoclusters. **c** | LAT can exist in sub-synaptic vesicles in addition to the plasma membrane. After TCR engagement, the LAT found in vesicles is phosphorylated and propagates TCR signalling. WASP, Wiskott–Aldrich syndrome protein.

engaged following TCR triggering and also how the movement of these molecules is laterally constrained on the cell surface. Despite limitations in the range of interactions that can be followed, which might not precisely mimic all of the receptor–ligand engagements that occur when a T cell interacts with an APC, novel data on the dynamics and the sequence of interactions that occur between various signalling molecules have accumulated and have enhanced our understanding of how T cell responses are both initiated and regulated.

The probability of signalling molecules interacting with each other is a function of their local concentration. The compartmentalization of molecules in the cell and their temporal regulation are now emerging as crucial factors that influence the signalling outcome of TCR triggering. One study that investigated the spatiotemporal organization of TCR signalling molecules at a systems level showed that the distribution of 30 chosen green fluorescent protein (GFP)-labelled signalling molecules, including ZAP70, LAT, protein kinase Cθ (PKCθ), ITK and PLCγ1, is different depending on the TCR signal strength[69]. These data show that the same TCR and signalling machinery can respond differently to varying levels of agonist peptide by regulating the spatiotemporal organization of signalling molecules.

A molecule of particular interest in this spatiotemporal control is ITK. T cells deficient in ITK show dramatically altered localization of molecules associated with TCR signalling, particularly at the T cell–APC interface[70]. For example, the recruitment of the small RHO-family GTPase cell division control protein 42 (CDC42) to the T cell–APC interface, which is crucial for regulating actin polymerization, is severely compromised in ITK-deficient mice. TCR signalling requires actin polymerization, and inhibition of this results in the termination of TCR signalling[71]. Rearrangement of the actin cytoskeleton is thought to govern several aspects of T cell activation, including the movement of TCRs into the cSMAC[58]; the formation of T cell–APC contacts[72]; and its function as a scaffold for the further assembly and stabilization of signalling complexes[73]. Mutations affecting ITK lead to decreased responses to TCR stimulation, particularly with respect to T cell proliferation and cytokine production[74]. In addition, ITK is an important link between LAT and integrin activation[75] (see below). If the regulation of actin polymerization is altered in ITK-deficient cells, this might affect the organization of scaffolds and signalling complexes, which might subsequently influence the routes by which the signals travel through the signalling cascades (that is, sideways and not downwards) and would thus affect the final outcome for the cell.

TCR signal diversification

Integrin signalling and its role in T cell activation. In addition to engagement of the TCR with peptide–MHC complexes, engagement of integrins plays an important part in the activation of T cells. Early reductionist approaches used planar lipid bilayers containing peptide–MHC complexes and intercellular adhesion molecule 1 (ICAM1), which is the ligand for LFA1, to stimulate T cells (reviewed in REF. 76). These early studies showed that, at T cell–APC contacts, the engaged TCR–peptide–MHC and LFA1–ICAM1 complexes are rapidly organized into discrete locations in the immunological synapse[58]. Initial contacts between T cells and APCs are relatively transient, as integrin receptors expressed on unactivated T cells have a low basal affinity that does not promote strong adhesion between the integrin and its ligand. However, this situation changes when TCRs are triggered, at which point inside-out signals to LFA1 are generated (FIG. 4). These inside-out signals increase the affinity of LFA1 for ICAM1 by inducing a conformational change in the integrin. This allows the formation of stable conjugates, which are required for the prolonged signalling needed for the full activation of the T cell.

Integrins were recently shown to influence microcluster dynamics and thus, potentially, the duration of TCR signalling complexes in T cells. Very late antigen 4 (VLA4; also known as α4β1 integrin) is upregulated on T cells after activation[77]. Co-stimulatory signals provided by the binding of VLA4 to its ligands enhance the signalling that is initiated by TCR engagement and have been shown to be dependent on the presence of SLP76 in the cells[78]. Mechanistically, VLA4 ligation enhances the persistence of SLP76 microclusters, and prolongs the interaction of SLP76 with ZAP70 and the TCR at the boundaries of mature signalling microclusters. The consequence of these prolonged interactions is increased phosphorylation of SLP76 and a decrease in retrograde actin flow, which is required for the centralization of SLP76 microclusters in the cSMAC. Centralization of microclusters is thought to promote the degradation of these T cell signalling complexes, and therefore, retention of microclusters away from the cSMAC would prolong TCR signalling. In this way, the upregulation of integrins, such as VLA4, might increase the sensitivity of activated effector T cells to previously encountered ligands[79]. However, increasing the sensitivity of T cells to foreign antigens risks lowering the threshold of activation in response to self molecules, with the associated risk of autoimmunity. In effector T cells, this is counteracted by the upregulation of negative regulators of TCR signalling, such as PTPN22 (discussed below), which help to maintain a balance that permits activation of T cell responses to agonist peptides but not to self peptides.

Coordination between integrin and TCR signals. The signals that regulate integrin activation following TCR stimulation are not fully understood but the small G protein RAP1 (also known as TERF2IP) is known to be an important player in this process (FIG. 4). RAP proteins have been implicated in the regulation of cytoskeletal rearrangements, in integrin-mediated cell adhesion and in intracellular trafficking[80]. Activation of T cells results in the enrichment of active GTP-bound RAP1 at the plasma membrane[81], and RAP1 has been shown to positively regulate T cell activation by controlling integrin activation[82] (FIG. 4). Very upstream signal transduction molecules such as LCK and ZAP70 are essential for inside-out signalling[83], and several studies have identified complexes containing RAP1, either RAPL (also known as RASSF5) or RAP1–GTP-interacting adaptor molecule (RIAM; also known as APBB1IP), SRC kinase-associated phosphoprotein of 55 kDa (SKAP55; also known as SKAP1), and adhesion- and degranulation-promoting adaptor protein (ADAP) as essential to this process[84,85]. In addition, SLP76 is an important component of TCR signalling to integrins. Therefore, a model was proposed in which phosphorylation of LAT recruits SLP76–VAV1 and ADAP, and results in the activation of RAP1 and the initiation of signalling to LFA1.

Inside-out signals
Integrins generate signal transduction in two directions, moving from the extracellular microenvironment into the cell cytoplasm (outside-in signalling) and from the cytoplasm out to the extracellular domain of the receptor (inside-out signalling).

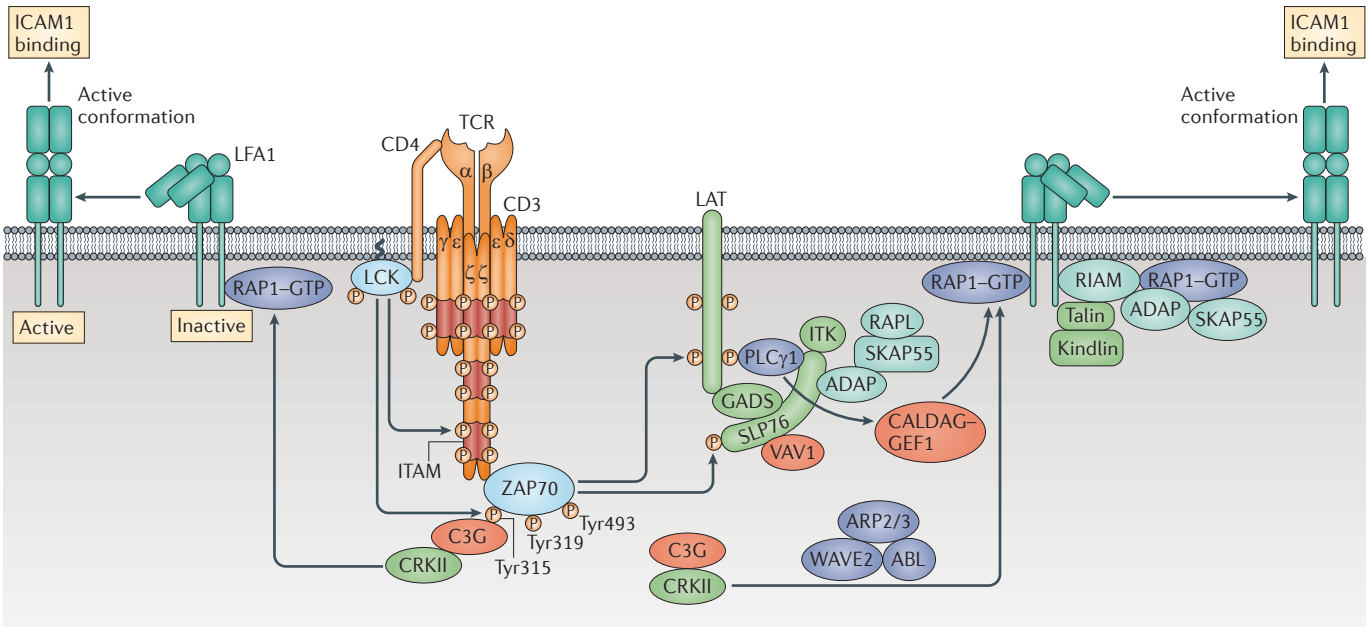

Figure 4 | **Mechanisms of TCR mediated inside-out signalling to integrins.** Upon T cell receptor (TCR) ligation, LCK phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs) of the TCR complex, leading to the recruitment of ZAP70 (ζ-chain associated protein kinase of 70 kDa) and its phosphorylation at multiple tyrosine residues, including Tyr315, Tyr319 and Tyr493. A crucial step in integrin activation is the conversion of the small GTPase RAP1 into its active GTP-bound form by guanine nucleotide-exchange factors (GEFs). Several different pathways have been proposed to deliver GEFs (shown in red) to the plasma membrane, leading to activation of RAP1, resulting in the activation of the integrin LFA1 through a process termed inside-out signalling. Once activated, ZAP70 phosphorylates linker for activation of T cells (LAT), resulting in the assembly of the LAT signalosome. Signalling downstream from phospholipase Cγ1 (PLCγ1), a component of the LAT signalosome, results in the activation of diacylglycerol (DAG)-regulated GEF1 (CALDAG–GEF1), which can activate RAP1. Alternatively, CRKII, an adaptor molecule which interacts with the GEF C3G, has been reported to bind to phosphorylated Tyr315 on ZAP70 after TCR stimulation and, in turn, leads to RAP1 and LFA1 activation. This pathway is independent of ZAP70 kinase function; rather, ZAP70 acts as a scaffold in this setting. CRKII–C3G can also be activated by the WAVE2 (WASP family member 2)–ARP2/3 (actin-related protein 2/3 complex)–ABL complex. RAP1 associates with various adaptor molecules forming modules consisting of RAPL, SKAP55 (SRC kinase-associated phosphoprotein of 55 kDa) and ADAP (adhesion- and degranulation-promoting adaptor protein), and RIAM (RAP1–GTP-interacting adaptor molecule), SKAP55 and ADAP. GADS, GRB2-related adaptor protein; ICAM1, intercellular adhesion molecule 1; ITK, interleukin-2-inducible T cell kinase; SLP76, SH2 domain-containing leukocyte protein of 76 kDa.

Although this might indeed be the major pathway of integrin activation for many T cell subsets, a recent study showed that some subpopulations of T cells, notably regulatory T (T_{Reg}) cells, can activate LFA1 through an alternative route. Au-Yeung *et al.*[86] generated a knock-in mouse by introducing a point mutation in the kinase domain of ZAP70 (ZAP70AS) that rendered the kinase activity of this protein readily inhibited by a small-molecule inhibitor when added to isolated ZAP70AS T cells *in vitro*[86]. As expected, inhibition of ZAP70 kinase activity in peripheral T cells completely abrogated the activation and effector function of all T cell subsets, with the surprising exception of T_{Reg} cells. Integrin activation has long been known to be an essential component of T_{Reg} cell function[87,88] but GTP-bound RAP1 is not impaired by the loss of ZAP70 enzyme activity in T cells[86]. Although recruitment of SLP76–VAV1–ADAP to LAT does not occur in the absence of the phosphorylation of crucial tyrosine residues in LAT, which depends on ZAP70 kinase activity, ZAP70 itself can act as an adaptor molecule for inside-out signalling. In the presence of the ZAP70 kinase inhibitor, LCK is activated following TCR ligation, which results in the phosphorylation of ZAP70 on two key residues, Tyr315 and Tyr319, in its interdomain region. These phosphorylated tyrosine residues bind to the adaptor molecule CRKII; CRKII associates with the GEF C3G and facilitates the exchange of GDP for GTP on RAP1 and hence inside-out signalling to LFA1. Thus, signalling to LFA1 downstream of TCR engagement can occur by several routes, at least one of which does not involve phosphorylation of the key adaptor protein LAT (FIG. 4).

These data provide a clear illustration of how signalling can diversify either through the generation of signalosomes based on dedicated adaptor proteins such as LAT and SLP76, or through an adaptor function associated with a kinase. This increases the potential for having discrete controls regulating different lineages of T cells. As T_{Reg} cells are essential for maintaining immune tolerance, it might be propitious to have them subject to subtly different control mechanisms from those that operate to restrain effector T cells.

Feedback that downregulates T cell activation

SFKs and the initiation of feedback loops. The threshold at which T cells become activated is determined by the avidity and/or the duration of TCR engagement. The formation of signalling microclusters and the initiation of signal transduction, such as the phosphorylation of key proximal signalling molecules, occur in seconds and minutes after TCR engagement, and, as measured using our relatively crude detection techniques, have generally decreased to undetectable levels by 10–20 minutes after TCR triggering. However, for full activation to occur — that is, commitment to T cell proliferation and to the development of effector function — there needs to be sustained signalling, which involves contact between the TCR and its ligands for several hours, as is observed *in vivo*[89]. Parameters such as the half-life of peptide–MHC complexes can affect cSMAC formation[90]. The premature interruption of TCR–peptide–MHC contacts leads to a failure of the T cell to progress to cell division, despite apparently normal early indicators of signalling[91]. These observations suggest that more than one threshold of activation needs to be exceeded for full T cell activation to occur. Moreover, these observations indicate the existence of multiple feedback loops that can terminate signalling at different stages of activation and that can re-instate the status quo (reviewed in REF. 10).

Phosphatases modulate SFK signalling. One important way in which the duration of signalling is regulated in T cells is through the action of phosphatases on the phosphorylated tyrosine residue in the active site of the SFK kinase domain. Precisely which phosphatases regulate this activity during TCR signalling has not been fully determined, but they include PTPN22, which interacts with CSK through a proline-rich PEST (proline-, glutamic acid-, serine- and threonine-rich) domain[92] and dephosphorylates the activating Tyr394 residue of LCK, downregulating its activity[93]. PTPN22 is upregulated in effector T cells; its absence increases the sensitivity of effector but not naive T cells to TCR stimulation[94,95], and it is implicated in the maintenance of T cell tolerance.

PTPN22 has recently increased in prominence because an Arg620Trp polymorphism in human *PTPN22* was found to be strongly associated with an increased risk for several autoimmune diseases[96–98]. Interestingly, mutant PTPN22 (Arg620Trp) has a greatly reduced interaction with CSK, although whether this polymorphism results in a gain- or loss-of-function allele is the subject of some controversy[99,100]. PTPN22-deficient mice have increased numbers of effector T cells[95] and an increased tendency to develop autoimmune inflammatory disease[94]. The absence of PTPN22 results in increased SFK signalling following TCR engagement on effector T cells, causing an increase in active GTP-bound RAP1 which, as discussed above, feeds into inside-out integrin signalling. Notably, despite showing an expansion of the population of effector T cells in a clean laboratory environment, PTPN22-deficient mice do not show evidence of autoimmunity, as they have T_{Reg} cells that are capable of regulating PTPN22-deficient effector T cells, unlike PTPN22-sufficient T_{Reg} cells[94]. Indeed, these PTPN22-deficient T_{Reg} cells have a marked upregulation of integrin activity and adhesion, which is consistent with the increase in the levels of active RAP1 in PTPN22-deficient T cells. This increased adhesion, together with greater production of the immunosuppressive cytokine IL-10, are likely to account for the enhanced regulatory function of PTPN22-deficient T_{Reg} cells. Interestingly, PTPN22 is a target of, and is downregulated by, forkhead box P3 (FOXP3)[101], the signature transcription factor required for T_{Reg} cell development and function. This indicates that suppression of PTPN22 is important for the function of T_{Reg} cells.

In contrast to RAS activity, RAP1 upregulation occurs only on the plasma membrane in fibroblasts and T cells[81] and, therefore, modifiers of SFK signalling, such as PTPN22, can influence whether signals are propagated down into the cell or sideways, by focusing signalling either downstream or laterally along the plasma membrane. These observations lend further support to the idea that the regulation of the branching of signal transduction pathways is distinct in different lineages of cells, such as T_{Reg} cells and effector T cells, and in this way might reduce the risk of T cell-mediated autoimmunity.

Concluding remarks

Classically, T cell signalling is described as going down from the TCR, via LAT, to activate the transcription factors that are required for T cell proliferation and for the differentiation of effector function. It is now clear that signalling also branches sideways via multiple adaptor domains and incorporates actin polymerization and cytoskeletal rearrangements. Signals are propagated to membrane-associated molecules, such as integrins, via the recruitment of small GTPases to the plasma membrane. In turn, integrin engagement influences the persistence of microclusters containing signalling molecules and, consequently, the extent of signal propagation. The regulation of these signalling cascades sets the threshold of activation of naive T cells so that they are not activated by self-peptide–MHC complexes and respond only to foreign-peptide–MHC complexes. Effector T cells are more sensitive to stimulation than naive T cells[79] and, therefore, have an increased potential for self-reactivity and to cause immune pathology. Negative regulators such as PTPN22 are upregulated in effector T cells to counteract this increased sensitivity to activation and to maintain immune tolerance. By contrast, T_{Reg} cells actively suppress PTPN22 upregulation and are even more effective suppressor cells when they lack PTPN22 expression entirely.

Under normal conditions, feedback loops exist to terminate or to limit T cell signalling, thus avoiding autoreactivity. Mutations in important regulators, such as LAT or the phosphatase PTPN22, perturb these feedback loops, perhaps by focusing the signalling cascade to inappropriate cellular locations, and thereby contribute to autoimmunity. Adopting a 'bigger picture' systems approach to identifying the spatiotemporal interactions between signalling components offers the exciting potential for gaining a better understanding of how these interactions are regulated, and might point to novel targets for therapeutic intervention to regulate T cell responses.

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Competing interests statement
The authors declare no competing financial interests.

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